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Regulation of Joint Destruction and Inflammation by p53 in Collagen-Induced Arthritis

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The role of the tumor suppressor p53 as a key regulator of inflammation was examined in murine collagen-induced arthritis (CIA), a model of rheumatoid arthritis. Wild-type DBA/1 mice develop progressive arthritis in this model, in which p53 expression and apoptosis are evident in the synovial cells. In contrast, the joints of $p53^{-/-}$ DBA/1 animals with CIA showed increased severity of arthritis using clinical and histological scoring methods with almost no apoptosis. Consistent with this, collagenase-3 expression and cytokine production (interleukin-1 and interleukin-6) in the joints of $p53^{-/-}$ mice with CIA were significantly greater than in wild-type mice. Anti-collagen antibody titers, however, were not different. Therefore, p53 expression occurs during inflammation and acts to suppress local inflammatory responses. Because mutations in p53 have been described in the synovial membrane of rheumatoid arthritis patients, the loss of p53 function in synoviocytes or other cells in the joint because of dominant-negative mutations might contribute to invasion and destruction of the joint in this disease. ***(Am J Pathol 2002, 160:123–130)***

Rheumatoid arthritis (RA) is a chronic inflammatory disease marked by hyperplasia of the synovial membrane and destruction of the extracellular matrix by the synovium. The invasive properties of rheumatoid granulation tissue (ie, pannus) and fibroblast-like synoviocytes (FLS) have led to the idea that partial transformation of synoviocytes contributes to the pathogenesis of RA. This hypothesis is supported by several characteristics of FLS, including anchorage-independent growth and the loss of contact inhibition by cultured FLS, increased telomerase activity in RA synovium, and oligoclonal expansion of FLS at sites of bone and cartilage erosions.[1,2] The most compelling evidence supporting this idea is the observation that RA FLS invade normal cartilage co-implanted into SCID mice whereas normal and osteoarthritis FLS do not.[3] Hence, rheumatoid synoviocytes are altered or transformed by their exposure to the inflammatory microenvironment, and these changes are major contributors to the destructive phase of the disease. The mechanism of abnormal synoviocyte biology in RA might be because, in part, of the presence of somatic mutations of the p53 gene in human RA synovium and cultured synoviocytes.[4–6] These mutations can be dominant-negative, indicating that there is loss of p53 function in these RA cells.[7]

The tumor suppressor gene p53 has well-established roles in cell-cycle control and apoptosis in response to DNA damage.[8] Such damage is found in rheumatoid synovium,[9] perhaps as a consequence of reactive oxygen and nitric oxide produced during inflammation. At the same time, p53 expression is increased in rheumatoid synovium.[10] Overexpression of p53 in inflammation is not unique to RA and is now known to occur in many other inflammatory conditions.[11] However, previous studies have not considered the function of p53 under these circumstances beyond its well-described response to DNA damage. We, therefore, hypothesized that p53 is an important homeostatic protein that has anti-inflammatory effects and that its expression will serve to down-regulate inflammation. To address this question, we examined the course of collagen-induced arthritis (CIA) in DBA/1 mice with homozygous disruption of the p53 gene. These studies showed that inflammatory arthritis was significantly greater in the $p53^{-/-}$ mice than in mice with functional p53 genes. The mechanism was related to decreased apoptosis along with enhanced synovial expression of cytokines and matrix metalloproteinases (MMPs).

Materials and Methods

Animals

$p53^{-/-}$DBA/1 mice were generated by successive backcrosses (more than eight) of male $p53^{-/-}$ B6.129S2-$Trp53^{tm1Tyj}$ (Jackson Laboratory, Bar Harbor, ME) × female DBA1. Most mice were used after the ninth to tenth

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generation of backcrossing. Genotypes were determined by polymerase chain reaction analysis of genomic DNA isolated from tail samples using the following three primers: x6.5 (ACAGCGTGGTGGTACCTTAT); x7 (TATACTCAGAGCCGCCT); and neo (CTATCAGGACATAGCGTTGG). Targeted mutation (knockout) alleles were identified by a 575-bp polymerase chain reaction product, whereas wild-type alleles gave rise to a 375-bp product. $p53^{+/-}$ DBA/1 or $p53^{-/-}$ males were bred with DBA/1p53$^{+/-}$ females to yield $p53^{+/+}$ DBA/1, $p53^{+/-}$, and $p53^{-/-}$ for the experiments described. To verify that the backcrossed mice were congenic at another locus, peripheral blood mononuclear cells were evaluated by fluorescence-activated cell sorting analysis using anti-I-A^q (clone KH116) and anti-I-A^b (clone AF6-120.1) antibodies (BD-PharMingen, La Jolla, CA). All mice ($p53^+$ and $p53^{-/-}$) were positive for I-A^q and negative for I-A^b. A C57BL/6 control mouse (H2b), negative for I-A^q and positive for I-A^b, was included as a staining control (data not shown).

Induction of CIA

Mice (6 to 8 weeks old) were immunized at the base of the tail with 0.1 ml of a solution containing bovine type II collagen (1 mg/ml) (Chondrex, Redmond, WA) in complete Freund's adjuvant. On day 21, 100 μg of type II collagen in 0.1 ml of phosphate-buffered saline (PBS) was injected intraperitoneally. Clinical arthritis scores were calculated using a semiquantitative scale of 0 to 4+ for each paw (hind paw: 0, no arthritis; 1, ankle swelling; 2, ankle and midfoot swelling; 3, ankle, midfoot, and metatarsal-phalangeal joint swelling; 4, ankle, midfoot, metatarsal-phalangeal joint, and digit swelling; scoring system for the forepaw was analogous; maximum score, 16 per animal). Four separate experiments were performed and the results for 13 $p53^{-/-}$ and 39 $p53^+$ (homozygous and heterozygous) mice were pooled. Mice were not genotyped until after the study was completed. All animals were handled in accordance with UCSD Animal Subjects Committee and USDA guidelines. No tumors were observed in any animals during the course of these experiments. Right hind paws from each animal were fixed in a 10% formalin solution, decalcified in 15% ethylenediaminetetraacetic acid-PBS, and embedded in paraffin for histological analysis. Five-μm sections were cut, mounted on glass slides, and stained with hematoxylin and eosin (H&E) or safranin O. The tissue was evaluated using a semiquantitative scoring system for synovial hyperplasia, cartilage erosion, extra-articular inflammation, and cartilage proteoglycan content on safranin O and H&E-stained sections (0 to 3+ per each of the four items; maximum score, 12 per animal), and the total histological score was compared between $p53^{-/-}$ ($n = 6$) and $p53^+$ mice ($n = 17$).

Fibroblast-Like Synoviocytes (FLS)

FLS from normal $p53^{-/-}$ and $p53^+$ mice were isolated and cultured as previously described.[12] Briefly, synovial tissues were obtained from microdissected ankle joints of the $p53^{-/-}$ and $p53^+$ mice. The tissues were minced under sterile conditions and incubated with 1 mg/ml of collagenase in serum-free Dulbecco's modified Eagle's medium (Life Technologies, Inc., Grand Island, NY) for 2 hours at 37°C, filtered through a nylon mesh, extensively washed, and cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 2 mmol/L glutamine, 50 μg/ml gentamicin, 100 U/ml penicillin, and 100 μg/ml streptomycin, in a humidified 5% CO_2 atmosphere. After overnight culture, nonadherent cells were removed, and adherent cells were cultivated in the same medium. At confluence, cells were trypsinized, split at a 1:3 ratio, and recultured in medium. FLS from passages 3 through 6 were used in these experiments.

Terminal dUTP Nick-End Labeling (TUNEL) Assay

Decalcified paraffin-embedded sections of CIA ankle joints (day 43) were processed for TUNEL assays as per the manufacturer's instructions (*In Situ* Cell Death Detection Kit; Roche, Mannheim, Germany). Briefly, the tissue sections were deparaffinized and rehydrated. Sections were then overlaid with 20 μg/ml of proteinase K and subsequently permeabilized with 0.1% Triton X in 0.1% sodium citrate. TUNEL reaction mixture was added and the tissue sections were counterstained with propidium iodide. The number of synovial $TUNEL^+$ cells/high-power field was counted in 5 to 10 fields for each coded slide.

Western Blot Analysis

$p53^{+/+}$ mice were immunized with type II collagen as described above. At various time points, groups of three to four animals were sacrificed, and joint tissues from left hind paws were collected and frozen until used. The frozen joint tissues were pulverized, and protein was extracted using RIPA buffer (50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate). The protein concentrations were determined with the DC Protein Assay kit (Bio-Rad, Hercules, CA). Equal amounts of protein samples (80 μg/lane) from pooled joint extracts at each time point were fractionated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane in transfer buffer (39 mmol/L glycine, 48 mmol/L Tris base, 0.037% sodium dodecyl sulfate, 20% methanol) at 100 mA for 2 hours. Differences in cellularity caused by progression of arthritis was normalized by loading similar amounts of protein in each lane. The membranes were blocked in Tris-buffered saline containing 0.1% Tween 20 and 5% dry milk for 1 hour at room temperature. p53 protein was detected with 1 μg/ml of FL-393, an affinity-purified rabbit polyclonal anti-p53 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), which recognizes murine, rat, and human p53 protein. After incubation with horseradish peroxidase-conjugated anti-rabbit IgG antibody (Roche), peroxidase

activity was detected using hydrogen peroxide as substrate and visualized by chemiluminescence.

Anti-Type II Collagen Antibodies and Cytokines

Serum was collected from mice on day 20 or 43 at the time of sacrifice and assayed for anti-mouse type II collagen antibodies using a commercially available enzyme-linked immunosorbent assay (ELISA) (Chondrex). Antibody titers are presented as arbitrary units compared with a known standard. For tissue cytokine assays, snap-frozen joints were homogenized in lysis solution (100 mmol/L potassium phosphate, pH 7.8, 0.2% Triton X-100, 1 mmol/L dithiothreitol). The volume of lysis solution was adjusted to 250 mg of tissue per ml. Tissue lysate was centrifuged, the supernatant was collected and the protein concentration was determined using the DC Protein Assay kit. Interleukin (IL)-6, tumor necrosis factor-α, and IL-1β were measured using a commercial ELISA according to manufacturer's instructions (R&D Systems, Minneapolis, MN). The values were normalized to protein concentration. The sensitivity of these assays was ~1 pg/mg of total protein. In other experiments, serum cytokines were also measured using the same ELISAs.

Northern Blot Analysis

Mouse limbs were cut just above and below the ankle or wrist and the skin was removed. Total RNA was fractionated on a 1.2% formaldehyde agarose gel and then transferred to a 0.45-μm nylon filter membrane. The blot was prehybridized in 50% formamide, 5× saline sodium phosphate-ethylenediaminetetraacetic acid, 5× Denhardt's solution, 1% sodium dodecyl sulfate, 200 μg/ml ssDNA, and 50 μg/ml tRNA. Mouse collagenase-3 (MMP13) or G3PDH cDNA was denatured and labeled by random-primed incorporation of ^{32}P-dATP (Random Primed Labeling Kit, Roche). The blot was hybridized overnight at 42°C, washed, and exposed to Kodak X-Omat AR film (Rochester, NY) with an intensifying screen at −80°C.

Statistics

Statistical significance was analyzed using the software package Statview 4.5 (Abacus Concepts, Berkeley, CA). Data are expressed as means ± SEM. Comparisons were performed using the unpaired Student's *t*-test or by analysis of variance. Differences were considered statistically significant when $P < 0.05$.

Results

p53 Expression in CIA

To determine the role of p53 in an animal model of chronic inflammation, we first examined its expression in CIA in DBA/1 mice. Western blot analysis was used to determine the levels of immunoreactive p53 in the joints of mice throughout the disease. As shown in Figure 1, p53 protein expression in joint tissues increased dramatically by day 20 after immunization and remained elevated through at least day 45.

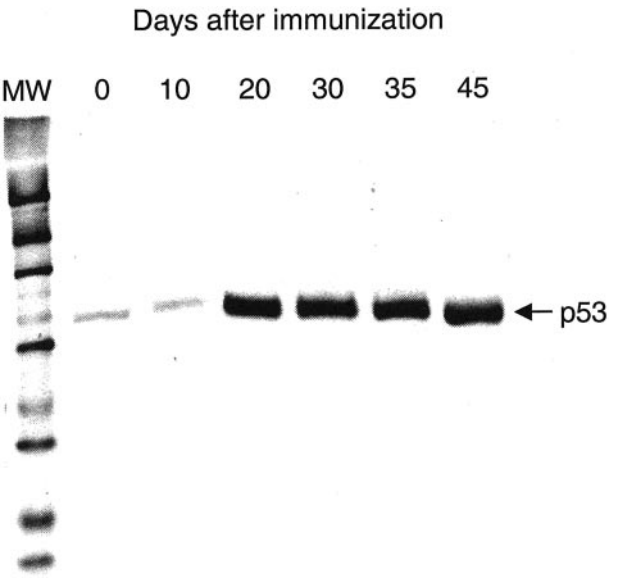

Figure 1. p53 expression in CIA. p53 protein levels in joint extracts were determined by Western blot analysis at various time points during CIA. Small amounts of p53 protein were detected in normal mice, but expression increased by day 20 (n = 3 to 4 animals per group).

Increased Arthritis Severity and Joint Destruction in p53$^{-/-}$ Mice

DBA/1 mice were bred with p53$^{-/-}$ animals and backcrossed for at least eight generations, and CIA was induced in p53^{+} (homozygous and heterozygous) and p53$^{-/-}$ littermates. No significant differences were observed between the p53$^{+/+}$ and p53$^{+/-}$ mice with any of the parameters evaluated, so the results for these two groups were pooled and referred to as p53^{+}. To determine whether loss of p53 function can contribute to the severity of chronic inflammation, we examined the clinical course of CIA in p53$^{-/-}$ mice. The mice were immunized with bovine type II collagen and clinical arthritis was measured using a semiquantitative scoring system. As shown in Figure 2, the severity of arthritis in p53$^{-/-}$ mice was not significantly increased in very early disease but was greater in the p53$^{-/-}$ mice compared with p53^{+} mice in late disease. The arthritis scores at the conclusion were 11.5 ± 1.0 for p53$^{-/-}$ mice and 8.1 ± 0.8 for p53^{+} animals ($P < 0.03$). The differences in mean scores indicates an increase in the number of joints involved. For instance, the average hind paw in p53^{+} mice had a score of ~2 (ankle and midfoot involvement only). However, the p53$^{-/-}$ mice had an average hind paw score of nearly 3, indicating involvement of the five metatarsal-phalangeal joints in each paw. Virtually all of the paws were involved in the mice by day 35 and were affected coordinately, so the differences in arthritis scores in late disease were not because of high joint counts in one limb with no arthritis in another limb of an individual animal. In addition to in-

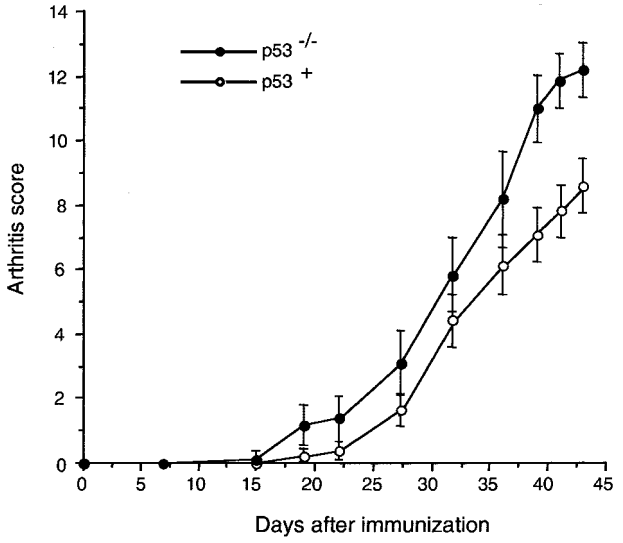

Figure 2. Arthritis scores in $p53^{-/-}$ with CIA. Clinical arthritis scores are shown for $p53^{-/-}$ ($n = 13$) and $p53^{+}$ ($n = 39$) mice with CIA. The $p53^{-/-}$ mice had higher arthritis scores than the $p53^{+}$ animals in late disease ($P < 0.03$ after day 36).

volvement of more joints in $p53^{-/-}$ mice, histological evaluation of the ankles revealed significantly greater synovial inflammation and cartilage and bone destruction in the $p53^{-/-}$ mice (Figure 3) ($p53^{-/-} = 10.2 \pm 0.5$; $p53^{+} = 6.2 \pm 1.0$; $P < 0.02$). Therefore, $p53^{-/-}$ mice with CIA had both a greater number of affected joints and increased severity of arthritis in the involved joints compared with $p53^{+}$ mice.

Anti-Type II Collagen Antibodies in CIA

To determine the mechanisms of increased arthritis and joint destruction in the $p53^{-/-}$ mice, serum anti-type II collagen antibodies were measured by solid-phase ELISA. However, there were no significant differences between the $p53^{-/-}$ and $p53^{+}$ mice at the end of the study (Table 1). Because clinical disease is, in large part, initiated and perpetuated by development of anti-type II collagen antibodies,[13] enhanced immune responses to collagen probably do not contribute to the differences in disease severity in the $p53^{-/-}$ mice.

Decreased Apoptosis in the Synovium of $p53^{-/-}$ Mice

Apoptosis is low in the joints of RA patients despite the presence of abundant DNA damage.[14] However, the joints of mice late in the course of CIA show $TUNEL^{+}$ synovial cells indicating apoptosis (Figure 4). As shown in Figure 4, apoptosis assessed by TUNEL was nearly absent in the joints of animals lacking p53 in late disease when $p53^{-/-}$ mice have more clinically active and destructive arthritis ($TUNEL^{+}$ cells were 2.83 ± 0.43 per field in $p53^{+}$ animals *versus* 0.60 ± 0.15 per field in $p53^{-/-}$ mice). Therefore, apoptosis in the synovia of mice with CIA is p53-dependent. Essentially no apoptotic cells were present in the synovia of unimmunized $p53^{+}$ DBA/1

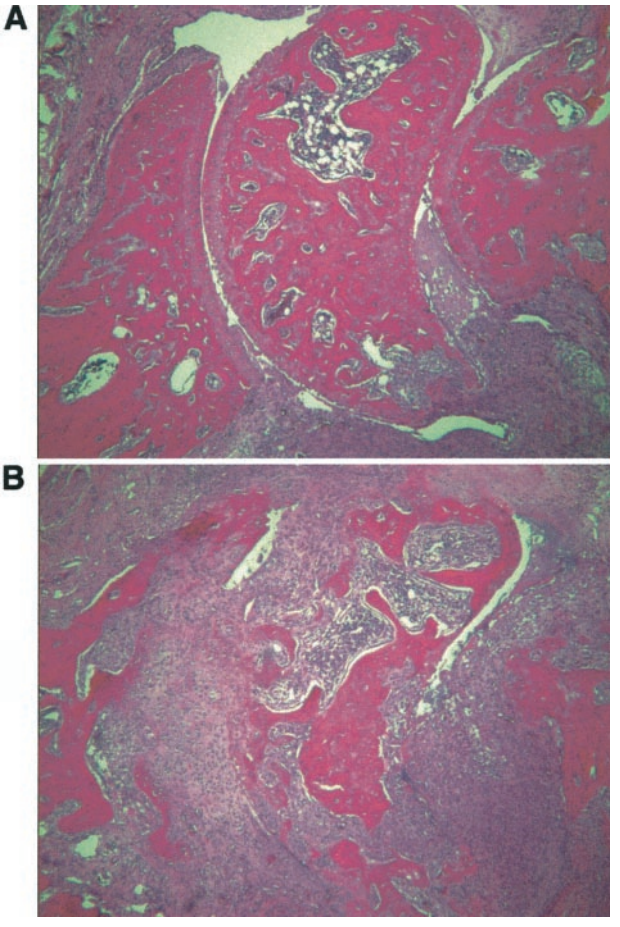

Figure 3. Synovial histology in $p53^{-/-}$ mice with CIA. Mice with CIA were sacrificed on day 43 and paraffin-embedded sections were stained with H&E. **A:** Joint from a $p53^{+/+}$ mouse with arthritis showing mild synovial hypertrophy and mild erosions. **B:** Joint from a $p53^{-/-}$ mouse with arthritis showing massive synovial inflammation and joint destruction. Synovial inflammation, bone erosions, and cartilage destruction were much more severe in the $p53^{-/-}$ mice.

Table 1. Antibody and Cytokine Levels in Collagen-Induced Arthritis

	$p53^{+}$	$p53^{-/-}$	*P* value
Anti-type II collagen antibody levels*			
Day 43	260 ± 48	299 ± 64	N.S.
Day 20	115 ± 13	69 ± 22	N.S.
Cytokine levels†			
Synovial IL-6			
Day 43	1.17 ± 0.62	14.72 ± 6.80	<0.05
Day 20	1.65 ± 0.69	1.80 ± 0.95	N.S.
Serum IL-6			
Day 43	41.8 ± 17.4	57.6 ± 29.3	N.S.
Day 20	85.9 ± 19.9	67.6 ± 5.6	N.S.
Synovial IL-1β			
Day 43	8.07 ± 1.72	69.5 ± 25.8	<0.05
Day 20	3.32 ± 1.68	3.98 ± 1.03	N.S.

*$\times 10^3$ U/ml; serum assayed for anti-type II collagen antibody by ELISA.

†Samples were assayed by ELISA. Synovial lysates = pg/mg total protein; serum = pg/ml; N.S.: not significant.

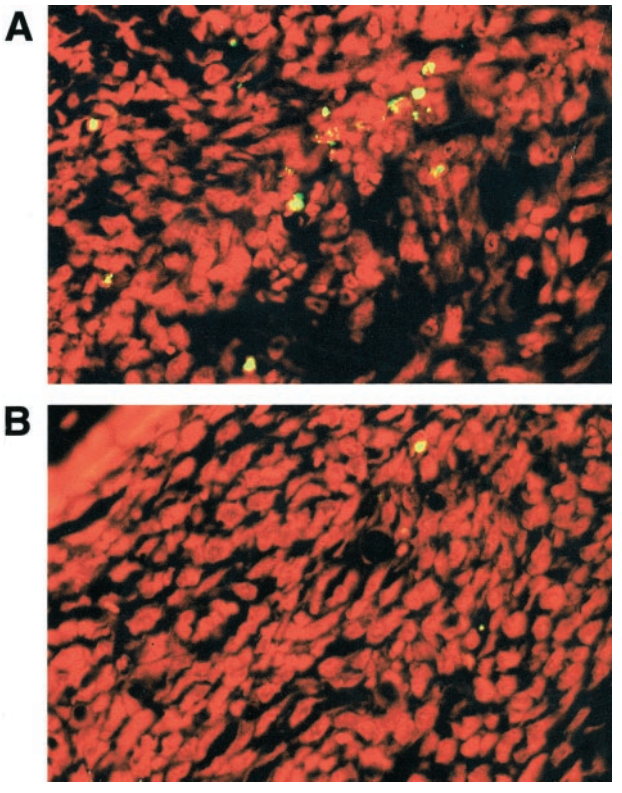

Figure 4. TUNEL-positive cells in $p53^{-/-}$ mice with CIA. Mice with CIA were sacrificed on day 43 and TUNEL assays were performed on paraffin-embedded sections. **A:** Synovium of $p53^{+}$ mouse with arthritis. **B:** Synovium of $p53^{-/-}$ mouse with arthritis. Note that apoptotic cells were detected in the synovium in the $p53^{+}$ mice but were rare in $p53^{-/-}$ mice.

mice.[15] We also did not observe $TUNEL^{+}$ cells in the synovia of $p53^{+}$ or $p53^{-/-}$ mice on day 20 after immunization, a time when both groups have similar arthritis scores. The level of apoptosis noted in the $p53^{+}$ mice during the late phase of disease (~1%) can clearly be a relevant determinant of tissue cellularity.[16]

Increased Synovial IL-1 and IL-6 Cytokine Expression in $p53^{-/-}$ Mice

Production of the proinflammatory cytokines IL-6 and IL-1β was markedly increased in the joints of the $p53^{-/-}$ mice with CIA on day 43 (Figure 5 and Table 1). Neither of these cytokines were increased in the joints of the $p53^{-/-}$ mice compared with $p53^{+}$ mice in very early disease (day 20), when the arthritis scores were similar to each other (Table 1). Tissue specificity for the increase in cytokine production was noted because serum levels of IL-6 were not significantly different in $p53^{-/-}$ and $p53^{+}$ mice (Table 1). Because p53 is known to suppress IL-6 gene transcription,[17] the mechanism of increased IL-6 production may be directly related to the absence of p53 or because IL-1 levels are increased.[18] The same lysates and serum samples were assayed for tumor necrosis factor-α; however, the concentrations were below the level of detection of the assay.

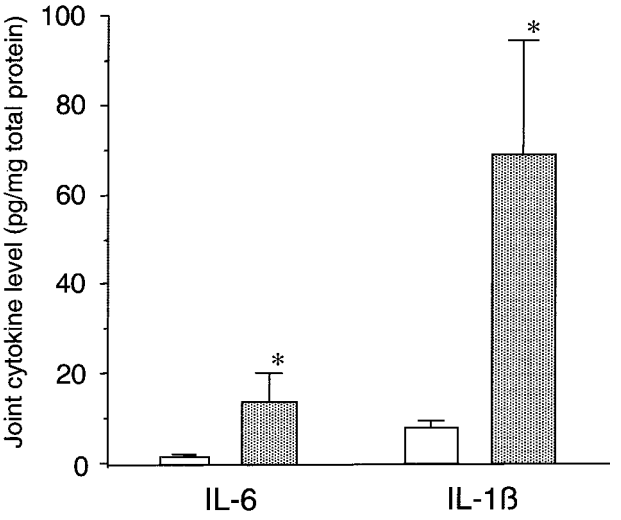

Figure 5. Increased IL-1 and IL-6 expression in the joints of $p53^{-/-}$ mice with CIA. Extracts of joints of $p53^{+}$ and $p53^{-/-}$ mice with CIA (day 43) were assayed for IL-1β and IL-6 by ELISA as described in Materials and Methods. Proinflammatory cytokine expression was significantly greater in the $p53^{-/-}$ mice (**shaded bar**) than $p53^{+}$ mice (**open bar**). *, $P < 0.05$.

Increased Synovial MMP13 Expression and FLS Proliferation in $p53^{-/-}$ Mice

Collagenase-3 (MMP13) expression levels might contribute to the increase in joint damage in $p53^{-/-}$ mice. mRNA samples from joint extracts were assayed for collagenase-3 gene expression by Northern blot analysis. Figure 6A shows that significantly higher levels of collagenase-3 mRNA were detected in the $p53^{-/-}$ mice compared with the $p53^{+}$ mice. Quantification of the differences shows

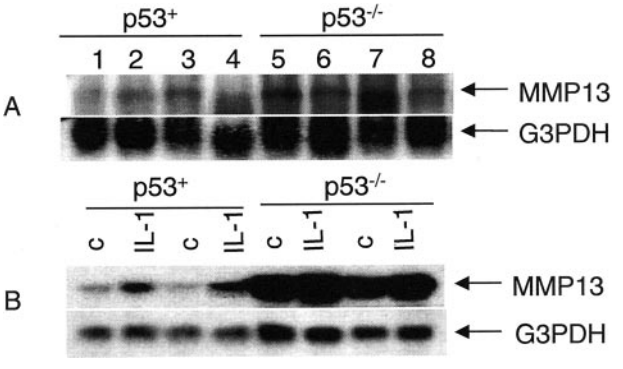

Figure 6. Regulation of MMP13 expression by p53 in CIA. **A:** Northern blot analysis of MMP13 expression in the joints of mice with CIA. MMP13 expression on day 43 is shown for four $p53^{-/-}$ and $p53^{+}$ mice (G3PDH mRNA expression is shown below). MMP13 expression was significantly higher in the joints of $p53^{-/-}$ mice by densitometry. Quantification of the mRNA expression shows that G3PDH-normalized MMP13 mRNA levels are 0.41 ± 0.08 in $p53^{+}$ mice and 0.80 ± 0.09 in $p53^{-/-}$ mice with CIA ($P < 0.02$). Individual normalized band densities are: $p53^{+}$ = 0.19, 0.39, 0.54, and 0.52; and $p53^{-/-}$ = 0.81, 0.70, 1.03, and 0.65. **B:** Northern blot analysis of MMP13 expression in cultured FLSs. Two $p53^{-/-}$ and $p53^{+}$ FLS lines were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and then stimulated with medium or 2 ng/ml of IL-1β for 18 hours. Basal and IL-1-stimulated MMP13 expression were much greater in the $p53^{-/-}$ FLS than in $p53^{+}$ FLS (G3PDH mRNA expression is shown below). Quantification of the mRNA shows that mean G3PDH-normalized MMP13 mRNA levels are 0.60 (medium) and 0.94 (IL-1-stimulated) in $p53^{+}$ FLS and 2.05 (medium) and 2.32 (IL-1-stimulated) in $p53^{-/-}$ FLS. Individual normalized band densities from left to right are: 0.75, 0.95, 0.44, 0.93, 2.03, 2.40, 2.07, and 2.23.

Table 2. p53 Effect on Synoviocyte Growth Rate

Day*	$p53^{+/-}$	$p53^{-/-}$
0	2.5†	2.5
2	2.6	2.8
5	3.2	7.0
7	5.4	9.6
10	7.4	12.0

*2.5×10^4 synoviocytes from $p53^{+/-}$ and $p53^{-/-}$ mice were cultured in 6-well plates on day 0. The number of cells in duplicate wells was counted on the indicated days, and the mean is shown in the Table.
†$\times 10^4$ cells/well.

that G3PDH-normalized collagenase-3 mRNA levels were 0.41 ± 0.08 in $p53^+$ mice and 0.80 ± 0.09 in $p53^{-/-}$ mice with CIA ($P < 0.02$). Furthermore, basal and IL-1-stimulated collagenase-3 gene expression was higher in cultured FLS from $p53^{-/-}$ mice compared with $p53^+$ mice (Figure 6B). Increased basal expression of MMP13 by cultured FLS grown in serum does not necessarily imply increased *in vivo* expression in the absence of inflammation. The situation is analogous to IL-6 (another p53-regulated gene) because the effect of p53 was not observed until late in the disease (Table 1). Previous studies have demonstrated that loss of p53 markedly increases basal IL-6 expression in cultured FLS.[7] Our findings are also consistent with recent studies that have shown that wild-type p53 suppresses collagenase gene transcription.[19] In addition, p53 can decrease synovial lining hyperplasia by inhibiting cell proliferation. As shown in Table 2, the growth rate of cultured synoviocytes from $p53^{+/-}$ mice was only half that of the $p53^{-/-}$ synoviocytes.

Discussion

Severity of arthritis, joint destruction, and articular cytokine expression are increased in $p53^{-/-}$ mice with CIA, which supports the hypothesis that p53 plays a key regulatory role in inflammation. We also demonstrated articular p53 expression in murine CIA as previously observed in rat adjuvant arthritis and patients with RA.[20,21] p53 protein distribution in synovial tissue typically includes both sublining and intimal lining cells. Therefore, the increase in CIA is likely because of expression in resident synovial lining cells (type B synoviocytes) as well as sublining infiltrating mononuclear cells. Elevated p53 has also been described in many other inflammatory diseases,[11] suggesting that p53 induction is a normal component of inflammation.

The protection afforded by p53 is probably due to suppression of effector mechanisms in CIA, because the production of anti-type II collagen antibodies was unchanged in $p53^{-/-}$ mice. These antibodies are critical to the initiation and severity of the disease, indicating that nonimmunological mechanisms are likely responsible for the increase in synovitis and joint destruction rather than antigen-specific responses.[22] In support of this hypothesis, p53 alters the cytokine balance in inflammation by suppressing proinflammatory cytokines such as IL-1 and IL-6 at the site of disease. The differences in cytokine balance are manifested in the late phase of CIA when synovial IL-1 and IL-6 production and clinical arthritis are significantly higher in $p53^{-/-}$ mice. Serum IL-6 is not increased, suggesting that the local inflammatory milieu is needed for excessive IL-6 induction in the absence of p53. Although it is difficult to separate the regulation of individual cytokines in the complex milieu of the joint, the p53-mediated decrease in IL-1β or IL-6 might result from a direct effect of p53[17,18] or secondary to other cytokine effects. Because IL-1 is a key regulator of joint destruction in animal models of arthritis, this could also contribute to increased bone destruction in the $p53^{-/-}$ mice.[23]

The present results support our hypothesis that p53 protects patients with RA and other inflammatory diseases. In this paradigm, the p53 gene is expressed in response to inflammation and genotoxic stimulation as a protective mechanism to induce cell-cycle arrest and apoptosis. Continued oxidative stress can eventually cause mutations in various genes, including p53, which supports cell for survival. Accordingly, apoptosis in the joints of mice with CIA is p53-dependent and indicates that a cell that lacking p53 function through mutation might have a growth advantage. Suppression of p53 function in human synoviocytes increases proliferation and invasiveness,[24] and mutant cells that arises in the RA joint could contribute to increased cytokine production. More recently, we have also demonstrated that loss of p53 function increases FLS invasion into cartilage in a SCID mouse model.[25]

Although the results vary, several groups have independently demonstrated somatic mutations in the p53 gene in synovial tissues of RA patients requiring joint replacement surgery.[4–6,26] The percentage of the p53 pool that is abnormal remains controversial, and generally varies between 5% and 30% of the total p53 mRNA. Disease stage or sampling error could account for some of these differences, because synoviocytes at sites of cartilage invasion are monoclonal or oligoclonal but are polyclonal when derived from nonerosive sites.[27] The p53 mutations in RA are generally G>A or T>C transitions, which is consistent with oxidative damage in the inflamed synovium.[11] We have also found that at least some of the mutations are dominant-negative and block the function of wild-type p53.[7] Therefore, cells expressing the dominant-negative p53 genes could be functionally similar to $p53^{-/-}$ cells even though the RA cells still possess a functional p53 allele.

CIA and perhaps RA are initiated by a systemic immune response, which plays an essential part in the joint disease. However, several studies suggest that subsequent events play a major role in joint destruction. For instance, anti-CD4 antibodies effectively suppress CIA when administered around the time of primary immunization, but at later times have little or no effect on the disease progression.[28,29] We propose that this immune response initiates the changes in the synovial microenvironment that induce apoptosis in $p53^+$ cells and thereby selects cells with mutations. Loss of p53 function in mu-

tant cells in the synovium[24] or because of inflammatory mediators such as MIF that suppress p53 function[30,31] not only protects them from apoptosis but also enhances invasiveness and collagenase expression.

Therefore, we suggest that p53 is induced in the rheumatoid joint and other sites of inflammation as a protective mechanism to arrest the cell cycle, suppress MMP expression, alter the cytokine network, and cause apoptosis. Because expression of secreted proteins such as MMPs, IL-6, and IL-1β is decreased by wild-type p53, dominant-negative mutations in RA could have significant local effects on inflammation and bone destruction even if a relatively small percentage of synovial cells harbor the mutant genes. In this way, a subpopulation of abnormal synoviocytes could enhance articular matrix destruction by releasing excess proteases and cytokines. In the murine model, all of the cells in the $p53^{-/-}$ mice lack this key gene, which could exaggerate the observed effect compared with RA. It is important to remember that the sequelae in RA would accrue throughout many years and is likely comparable to the highly compressed CIA model. The loss of p53 function in RA, as in CIA, could then lead to a more aggressive disease, especially at the invasive front of pannus that erodes the extracellular matrix. Novel therapeutic approaches to induce apoptosis or de-activate partially transformed synoviocytes could be beneficial in RA by helping restore synovial homeostasis.

Although overexpression of p53 in tissues was originally thought to be a surrogate marker for mutations, many studies have demonstrated wild-type p53 in human inflammatory diseases, including RA, inflammatory osteoarthritis, reactive arthritis, inflammatory bowel disease, psoriasis, *Helico pylori* infection, and many others.[11] We have also shown the same phenomenon in animal models of arthritis (adjuvant arthritis in rats[21] and, now, in CIA in mice). Hence, p53 induction is a general phenomenon in inflammation and it functions as a regulatory molecule that modulates normal inflammatory responses. Our observations suggest that methods to enhance p53 function or expression will have beneficial effects in many inflammatory diseases.

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